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Retrospective Observational Study of *Salmonella* Typhi and their Antimicrobial Susceptibility Pattern in a Tertiary Care Hospital

Rehana Razzak Khan[1*], Sourav Debnath[1], SM Ali Ahmed[1], Md. Saiful Islam[2], Ismet Nigar[1], Shaheda Anwar[1]

Abstract

Background: *Salmonella enterica* subspecies *enterica* serotype Typhi (*S.* Typhi) is mainly responsible for enteric fever. it poses a serious threat to public health in underdeveloped nations like Bangladesh. The growing antibiotic resistance observed in their management necessitates periodic monitoring of susceptibility trends to support therapeutic care at both the national and local levels. Furthermore, this will enable the planning of antibiotic recycling whenever feasible.

Objective: To provide a comprehensive understanding of how antibiotic susceptibility has evolved over time, this study aimed to identify *Salmonella* Typhi and to determine their demographics, seasonal fluctuations, and antibiotic susceptibility.

Methods: This retrospective study was conducted in the Department of Microbiology and Immunology, Bangladesh Medical University, Dhaka, Bangladesh, between July 2023 and June 2024. A total of 6572 blood samples were collected in an automated blood culture bottle, and the bacterial profile was retrieved using an automated BACT/ALERT 3D System and BD BACTEC FX continuous monitoring system. The collected blood sample was processed, and full identification of the organism and antimicrobial susceptibility was conducted by using the VITEK 2 Compact Lab automated system and Kirby Bauer disk diffusion methods per the National Committee for Clinical Laboratory Standards guidelines.

Results: A total of 6572 blood samples were collected, of which 530(8.06%) showed bacterial growth. Among them, number of total isolated *Salmonella* Typhi was 105(19.8%). The majority were between the age group 21-30 years old with female predominance and the highest prevalence occurred from November to the month of June. *S.* Typhi showed highest resistance to azithromycin (75.2%), maximum sensitivity to ceftriaxone and cefixime (100%; MIC ≤1 µg/ml and ≤ 0.25 µg/ml respectively) followed by chloramphenicol (88.57%; MIC ≤ 8 µg/ml) cotrimoxazole (81.90%; MIC ≤ 20 µg/ml) and ampicillin (64.76%; MIC ≤ 8 µg/ml). However, the fluoroquinolone class of antibiotics, nalidixic acid (74.4%; MIC 32 µg/ml) and ciprofloxacin (72.4%; MIC 1µg/ml) showed higher resistance.

Conclusion: The results of this investigation highlighted that the cephalosporin class of antibiotics is still 100% effective, and first-line antibiotics are becoming less resistant suggesting that it may be reintroduced as an empirical treatment for enteric fever. Prescription practices should be changed, and the usage of antibiotics without prescription should be reduced. These findings highlight the need for antimicrobial stewardship programs and the prudent use of antibiotics in Bangladesh's tertiary care hospitals.

Affiliation:

Department of Microbiology and Immunology, Bangladesh Medical University, Dhaka, Bangladesh. Department of Laboratory Medicine, Bangladesh Medical University, Dhaka

***Corresponding author:**
Rehana Razzak Khan, Department of Microbiology and Immunology, Bangladesh Medical University, Dhaka, Bangladesh.

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Introduction

The bacteria *Salmonella enterica* serovar Typhi is the cause of typhoid fever [1] is a major global health issue, particularly in South Asian low- and middle-income nations [2] which account for around 70% of all cases worldwide [3]. Typhoid has killed over 110,000 people worldwide, according to the Global Burden of Disease survey, with almost 9 million cases reported each year [4-5]. Children and young adults are primarily affected because they are either immunocompromised or have been exposed to fecal bacteria at high levels [2]. According to a number of studies, Bangladeshi street food is tainted with bacteria, and raw or undercooked food and contaminated water include *Salmonella* Typhi, which may spread quickly and cause illnesses like typhoid [6,1]. According to South Asia's overall sex discrepancies in enteric fever reporting, men made up the majority of cases (59%) [7]. In many Asian and African nations, enteric fever frequently recurs around the same time of year and exhibits a seasonal pattern [8]. In Bangladesh, Nepal, and Cambodia (South and Southeast Asia), incidence peaks around May to October [8]. Weather conditions may have an impact on their transmission [9].

Typhoid fever is difficult to distinguish from other causes of febrile illnesses, compounded by limited laboratory services in some low- and middle-income countries, making specific diagnosis and appropriate antimicrobial treatment challenging in routine practice [10,11]. A global concern is *Salmonella* Typhi's antimicrobial resistance (AMR) [12]. Historically, the first-line antibiotic therapies for typhoid fever were trimethoprim-sulfamethoxazole, ampicillin, and chloramphenicol [13]. However, during the late 1980s and early 1990s, reports of multidrug-resistant (MDR) *Salmonella* Typhi which is classified as resistant to these three first-line drugs were coming from Pakistan, India, and other southern and Southeast Asian nations [14,15]. Ciprofloxacin subsequently emerged as the preferred medication; nevertheless, resistance swiftly emerged, initially in 1991 and then in an outbreak in 1997[16,17]. The usage of third-generation cephalosporins, macrolides, and carbapenems to treat typhoid fever has increased since the advent of MDR *Salmonella* Typhi and MDR with fluoroquinolone resistance were discovered [13]. Hence, *Salmonella* Typhi is a WHO priority pathogen for AMR surveillance [18]. Understanding antimicrobial sensitivity and resistance patterns against the main types of antibiotics used to treat typhoid fever is the main goal of our research. Because research from various nations has demonstrated a varying pattern of susceptibility to conventional drugs, our study aimed to determine demographic information, and seasonal fluctuation, and monitor antibiotic susceptibility patterns that are therapeutically significant.

Materials and Methods

Study design

A retrospective review of the laboratory data of blood samples obtained from the inpatient and outpatient departments of Bnagladesh Medica University between July 2023 and June 2024 was carried out by the Department of Microbiology and Immunology.

Study population

A total of 6572 blood samples were collected during that period. Reports of *Salmonella* Typhi from blood cultures were retrieved and analyzed. Comprehensive data regarding demographic data, seasonal variation, laboratory results of bacterial isolation, and susceptibility patterns were collected from the Laboratory specimen logbooks using the standard data collection form.

Laboratory procedures

Sample collection

Adult and pediatric BACT/ALERT blood culture bottles were used. About 8-10 ml blood/bottle for adults and 3-5 ml blood/bottle for pediatric patients were collected in the blood culture bottle, labeled properly, and transported to the Microbiology laboratory without delay for bacteriological examination.

Organism isolation

All the samples were incubated using an automated BACT/ALERT 3D System and BD BACTEC FX continuous monitoring system. When the system indicated growth, blood agar and MacConkey agar media were used as solid culture media to isolate the organism. Organisms were identified based on morphology, culture characteristics, and biochemical reactions according to standard microbiological techniques.

Antimicrobial susceptibility

Culture showing significant growth of organisms was identified to species level and AST was performed by VITEK 2 compact GN cassette AST-N405 card (bioMérieux, Inc., Durham, NC) according to manufacturer's instructions. The turbidity was adjusted to 0.5 as per McFarland standard by Densi CHEK Plus. The suspension was inoculated into the VITEK 2 compact with a GN cassette AST card. According to VITEK 2 compact system special software, interpretation of the results was performed as explained by the manufacturer's instructions. The result was interpreted according to CLSI guidelines (Clinical Laboratory and Standards Institute) [19]. For quality control VITEK 2 GN QC for ID validation card uses.

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The antibiogram was obtained by VITEK 2 MIC system of the following antibiotics ampicillin (MIC ≤ 8µg/ml), cotrimoxazole (MIC ≤ 40µg/ml), ciprofloxacin (MIC ≤ 0.0625µg/ml), Nalidixic acid (MIC ≤ 2µg/ml) ceftriaxone (MIC≤ 1µg/ml), Cefixime (MIC≤ 0.25µg/ml) chloramphenicol (MIC ≤ 8µg/ml). All the isolates were also tested for antimicrobial susceptibility on Muller Hinton Agar (HI Media, India) by Kirby Bauer disc diffusion method, according to the Clinical Laboratory Standard Institute (CLSI) guidelines [19]. The following antibiotics were used for *Salmonella spp*: amoxicillin (10µg), ciprofloxacin (5µg), nalidixic acid (30µg), ceftriaxone (30µg), trimethoprim-Sulfamethoxazole (1.25/23.75µg) cefixime (5 µg). And additional azithromycin (30µg) for *Salmonella* Typhi. All the antibiotic disks were commercially purchased from Bio maxima, Poland. *Salmonella* Typhimurium ATCC 14028 was included as a quality control strain of antimicrobial susceptibility testing.

Data analysis

Data were cleaned manually, entered, and analyzed by using SPSS version 24 software. The statistical analysis used in the study was descriptive and did categorical data analysis, Frequency and percentage were examined for categorical independent variables. Results were presented through graphs and tables.

Results

A total of 6572 automated blood samples were collected of which 530 (8.06%) yielded bacterial growth (Table I)

Table 1: Frequency of Bacterial isolates in blood sample (n=6572)

Culture	Frequency	Percentage (%)
Growth	530	8.06%
No Growth	6042	91.93%
Total	6572	100%

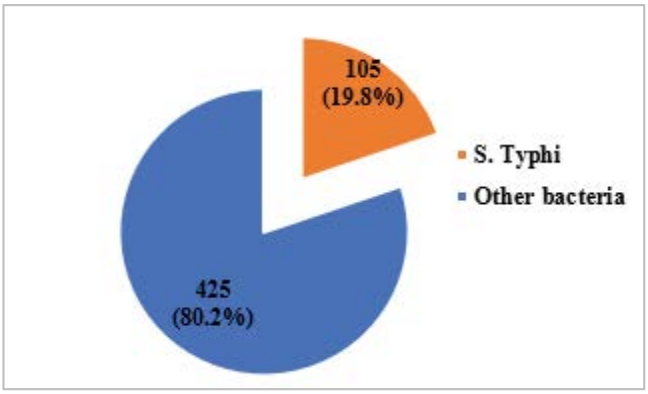

Figure 1: Distribution of bacterial growth among culture-positive cases (n=530)

Among the 530 bacterial growth, *Salmonella* Typhi were 105(19.8%) and other bacteria were 425(80.2%).

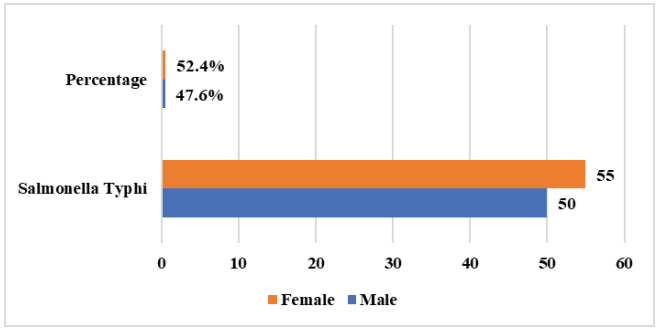

Figure 2: Distribution of the patients according to gender

In the present study out of 105 culture-positive *Salmonella* Typhi cases, 50 (47.6%) were males and 55(52.4%) were females showing almost the same proportion a little inclined towards females (Figure 2)

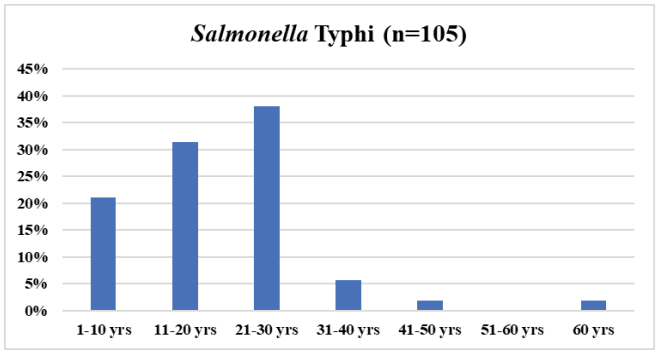

Figure 3: Age-wise distribution of culture-positive cases of *Salmonella* Typhi (n=105)

In the present study, out of 105 positive cases, (40, 38.1%) were from the age group 21-30 years followed by (33, 31.4%) from the age group 11-20 years, and (22, 21%) from age group 1-10 years. Only (6, 5.7%) were from 31-40 years and 41-50 years and more than 60 years (2, 1.9%) cases were found in each group. No patient was in the age range of 51–60 (Figure 3)

S. Typhi was isolated from blood cultures every month, with the greatest number of cases occurring from November to June with peaks in November, January, March, and June, Figure 4 provides an illustration of this.

The antibiotic susceptibility pattern of the 105 *Salmonella* Typhi isolated from blood sample is shown. (Table 2). MIC break point and zone diameter in the disk diffusion method were used according to CLSI guidelines. In the present study, the isolated *Salmonella* Typhi showed higher resistance to azithromycin (75.2%,), 74.4% of isolates showed a resistance to the nalidixic acid with there being an average MIC

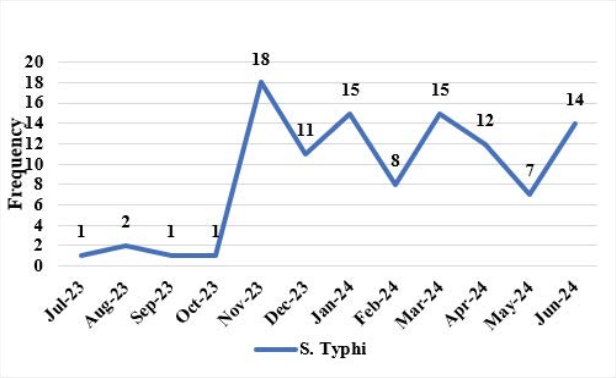

Figure 4: Month-wise distribution of cases of *Salmonella* Typhi (n=105)

≥32 µg/ml and 72.4% of isolates showed resistance to the ciprofloxacin with their being average MIC ≥1 µg/ml. Whereas none of the isolates showed resistance against ceftriaxone with a being an average MIC≤1 µg/ml and cefixime MIC≤ 0.25 µg/ml. Higher susceptibility against ampicillin 64.76% with an average MIC ≤8 µg/ml. However, it exhibited the highest susceptibility against chloramphenicol 88.57% with an average MIC ≤ 8 µg /ml and Cotrimoxazole 81.90% with average MIC ≤ 20 µg /ml. There are no intermediate-sensitive isolates in our study.

Table 2: Antibiotic susceptibility pattern of *Salmonella* Typhi to different antibiotics (n=105)

Antibiotic Class	Antibiotics	Sensitive (%)	Resistant (%)
Penicillin	Amoxicillin/ Ampicillin	68(64.76%)	37 (35.2 %)
Chloramphenicol	Chloramphenicol	93(88.57%)	12 (11.4%)
Sulfonamides	Trimethoprim-Sulfamethoxazole	86(81.90%)	19 (18.1%)
Cephalosporin	Ceftriaxone	105(100%)	0 (0%)
	Cefixime	105(100%)	0 (0%)
Fluoroquinolones	Nalidixic acid	27 (25.71%)	78 (74.4%)
	Ciprofloxacin	29(27.61%)	76 (72.4%)
Macrolide	Azithromycin	26 (24.76%)	79 (75.2%)

Discussion

In Bangladesh and other impoverished countries, enteric fever remains a serious health concern. These cases were mostly caused by *S.* Typhi bacteremia with high incidence rates linked to poor sanitation and unsafe food and water, particularly impacting children and adolescents. The bacterial isolation rate in this study was 8.06%, which is consistent with the Meiring et al. study which reported an isolation rate of 9.2% in Dhaka [20] also 10-year retrospective research conducted in Bangladesh found that the isolation rate ranged from 10.7 to 17.3% [21]. This similarity might be due to the availability of the latest automated systems. However, the isolation rate was 3% in Bangladesh and 0.53%, 4.1%, and 2.2% in India, Nepal, and Pakistan, respectively, as reported in Barkume et al.'s (2018) multiphase surveillance study for the Surveillance of Enteric Fever in Asia Pacific (SEAP) [7]. This difference is due to the different institutions' employment of varied sample collection techniques, and culture techniques. Among the culture-positive cases, *S.* Typhi was isolated (19.8%) with 52.4% cases in females and 47.6% in the male in the current study. The result of isolation in this study almost agreed with Asghar et al. (2024) study which reported 12% [22] and Khudhair et al. (2020) found 52% were female and 48% were male from their total isolate [23] also several studies reported female predominancy [24,25].

Typhoid fever was more common in women, and this was linked to a lack of knowledge, poor hygiene, a lack of education, and the use of tainted drinking water [23]. However, the Barkume et al. surveillance study reported the sex of the majority of participants were male [7]. Whether any factors contribute to the sex-specific vulnerability of enteric fever patients can be ascertained by additional research. The results of this study have also revealed that the higher incidence of infection with *S.* Typhi in adults 40(38.1%) between the age group (21-30) years and 33(31.4%) in the age group (11-20) years and then in children the 22(21%) between age group (1-10) years. In adults, the highest occurrence of infection with *S.* Typhi was concurrent with the findings of different studies [23,26,27]. Adults are particularly at risk since they make up the majority of the workforce and engage in more outside activities in poor nations, which allows them to consume cool water and undercooked or street cuisine on hot days.

A similar survey in Quetta reported infection incidence in (11-20) years age group seem to agree with our study [28]. This infection is brought on by school-age children's and college-going students' unsanitary behavior, poor hygiene practices, or developing a habit of consuming local street foods as it is more affordable and convenient in developing nations like Bangladesh. In children, (1-10) years also have an incidence of infection which correlates with several studies [22, 23,27]. This is due to weaker immune systems in growing children [22.23]. Evidence from past studies that shown environmental variation in the risk of infection between children and adults is emphasized by Akullian et al [2]. In the present study, throughout the year *S.* Typhi isolated with an increased peak in November, January, March, and June. Consumption of inadequately preserved food or previous travel can cause wintertime enteric fever. Furthermore, cold air weakens immunity, which makes fighting off diseases

more challenging. The peak of the summer dry season in our study is consistent with other studies showing that rising temperatures would encourage the growth and reproduction of *Salmonella*e, lead to water scarcity and pollution, encourage the purchase of prepared foods or barbecues during warmer weather, and use contaminated water sources [27, 29]. Numerous studies have demonstrated a favorable relationship between temperature and foodborne illnesses such as enteric fever in a variety of geographic contexts [30–31].

Drug-resistant typhoid fever cases are on the rise, which concerns not just for our region but also for the possibility of an international outbreak. *Salmonella* Typhi's azithromycin resistance was initially discovered in Tanmoy et al. studies in 2013, but it remained sporadic, with only 1% of isolates showing resistance in 2022 [31]. The consumption of the drug on the other hand increased [32]. It is rather alarming that 75.2% of *Salmonella* Typhi in our study were resistant to azithromycin. This is consistent with a study conducted in Bangladesh that indicates an increasing effective population size of isolates that are resistant to azithromycin [33]. High consumption of the drug may exert selective pressure fostering resistance determinants in the gut [32]. But according to a different study, azithromycin has the potential to be a good therapeutic option with a noteworthy sensitivity of 90.40 to 95% [34–37]. This supports the WHO's identification of azithromycin as a substitute treatment for MDR typhoid fever, especially in areas where resistance to traditional medication is high [38].

In the present study, it was shown that low susceptibility to nalidixic acid (74.4%; MIC 32 µg /ml) and ciprofloxacin (72.4%; MIC ≥1 µg /ml) was a major concern. This is consistent with the Singh et al. study which reports nalidixic acid (50%; MIC ≥ 32 µg /ml) and ciprofloxacin (76.8%; MIC ≥ 1 µg /ml) and Biswas et al reported 100% resistance to fluoroquinolone with average MIC of 2 µg/ml in case of ciprofloxacin and ≥ 32 µg /ml in case of nalidixic acid. [39.40]. This is consistent with several other studies where the majority of isolates in all countries were resistant to fluoroquinolones [7,31,34]. It suggests that these medications are being abused and used irrationally to treat many other non-specific illnesses. In the present study, has shown 64.76% sensitivity to ampicillin (MIC ≤ 8 µg/ml) and 81.90% to Cotrimoxazole (MIC ≤ 20 µg/ml) and chloramphenicol 88.57% with an average (MIC ≤ 8 µg/ml) which is consistent with the study by Singh et al. who reported more than 90% susceptibility to ampicillin (MIC ≤2.0 µg/ml) and 91.30% (MIC≤ 20 µg/ml) against cotrimoxazole and they did not mention about chloramphenicol [39]. This decrease in resistance may be the consequence of fewer doctors prescribing first-line antibiotics and a drop in usage, especially of cotrimoxazole. Few national and international study shown 95-100% sensitivity against *Salmonella* [41,42]. However, Qayyum et al. reported less sensitivity to chloramphenicol due to the discontinuation of its serious side effects like bone marrow suppression [38]. In our present observation, we did not find any MDR *Salmonella* Typhi cases. Whereas Khan et al (2024) in Pakistan, showed a high resistance rate to ampicillin (81.40%) and chloramphenicol (90.2%) respectively, and did not mention cotrimoxazole they also mention MDR cases [34].

The current study has shown 100% sensitivity to ceftriaxone (MIC ≤1 µg/ml) and cefixime (MIC ≤ 0.25 µg/ml). It consistent with several studies from Central Asia and Pakistan [40,41]. Singh et al reported, 93.47% sensitivity to ceftriaxone (MIC≤1µg/ml) [39]. But between April and September 2024, Hooda et al. found 47 instances of ceftriaxone-resistant *Salmonella* Typhi in Bangladesh, in addition to a few isolated cases that had previously been documented in Bangladesh and India [35,43-45]. Public health professionals must monitor for the introduction and spread of ceftriaxone-resistant *Salmonella* Typhi because ceftriaxone empirical treatment is so prevalent in South Asia [46]. However, Biswas et al reported 20% resistance to cefixime in 2019 [40]. The most common cause of antibiotic resistance in enteric fever is the improper, overuse, and abuse of antimicrobial medications. So medical professionals should consider this before giving antibiotics. However, the overall results could not accurately represent the nationwide situation of this endemic infection.

Conclusion

According to the current study, Adults and school-going children between the ages of 11-20 years are primarily afflicted by enteric fever, which is endemic throughout the year with a peak in November, January, March, and June. Reintroducing first-line antibiotics to treat this infection is one potential antibiotic stewardship strategy that we recommend in order to monitor the AMR pattern in enteric fever cases and reevaluate before prescribing azithromycin as its resistance increases in Bangladesh. Third-generation cephalosporins require close monitoring. According to these findings, treatment procedures must be modified, and quick action is needed to stop antibiotic overuse. Regularly assessing the microbiological profile is crucial to ensuring effective treatment of enteric fever cases, particularly given the growing issue of drug resistance.

Limitation

We were unable to differentiate between samples from patients outside of our hospitals and those from our hospitals due to the retrospective nature of the study. In addition to age and sex, we were unable to collect data regarding the patient's clinical manifestation or any other characteristics. A clinical relationship was not possible.

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Conflict of interest

The authors declare no conflicts of interest regarding the publication of this paper.

Authors Contributions

All authors contributed equally to this work.

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